same amino acid composition as the wild-type parentals of *S. cerevisiae* studied in this work.[9]

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Isotope-edited nuclear magnetic resonance: novel methodologies for investigating metabolism

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Abstract: While the use of NMR and stable isotopes in metabolism studies is hardly new, it is only recently that isotope-edited NMR spectroscopy has been applied in kinetic studies of glyphosate metabolism of soil microbes. NMR can detect multiple species simultaneously and non-destructively, yielding valuable information on structural identification of metabolites.

Triple Resonance Isotope EDited spectroscopy (TRIED), [^{2}H]NMR, and [^{2}H–^{13}C] INEPT (Insensitive Nucleus Enhancement through Polarization Transfer) are three isotope-edited techniques which have been used in combination to examine the microbial degradation of glyphosate (*N*-phosphonomethylglycine). Using ^{13}C- and ^{15}N-labeled glyphosate, TRIED can detect multiple metabolites in crude matrices at submicrogram levels, an improvement over earlier techniques where milligrams were needed. It can detect 500 nanograms of ^{13}C–^{15}N-labeled compound in a crude sample (1 : 1400 mass ratio), only a few hours work being required. [^{2}H]NMR and [^{2}H–^{13}C]INEPT were also used as complementary techniques to further examine metabolites whose ^{13}C–^{15}N bond has been cleaved.

The three-isotope-edited methods produced results consistent with both radioactivity and HPLC analyses. Accordingly, we are able to detect minute levels of metabolites in the presence of complex mixtures, minimizing the costs and time of sample purification.

Keywords: NMR; isotope-edited NMR; herbicide metabolism; microbial soil isolates

Triple Resonance Isotope EDited spectroscopy (TRIED), [^{2}H]NMR, and [^{2}H–^{13}C]INEPT (Insensitive Nucleus Enhancement through Polarization Transfer) form a powerful arsenal of isotope-edited techniques that can be used to study metabolism in extracts from biological systems. All three methods circumvent the use of radioactive labels. TRIED was developed to detect and examine minute levels of glyphosate (*N*-phosphonomethylglycine) metabolites in microbial soil isolates.[1] It uses stable isotopic labeling (^{13}C and ^{15}N), and allows the simultaneous detection of multiple metabolites in crude matrices at submicrogram levels with high signal-to-noise (s/n) ratios and nearly complete suppression of unwanted signals. Both [^{2}H]NMR and [^{2}H–^{13}C]INEPT allow continued metabolic studies of labeled compounds beyond the point where the ^{13}C–^{15}N bond has been broken. The pathways of glyphosate metabolism by LBAA, one strain of *Ochrobactrum anthropi*, have been studied using isotope-edited experiments. No detectable levels of glycine or sarcosine were observed in media or lysates, leaving the aminomethylphosphonate (AMPA) pathway as the means of glyphosate metabolism. Other metabolites produced included *N*-methylAMPA, acetylAMPA, glyphosate esters, and *N*-methylacetamide.[2]

TRIED is patterned after triple-resonance techniques used in NMR measurements of ^{13}C–^{15}N labeled proteins. Coherence originates as proton magnetization, which is transferred from the protons to their adjacent carbons, and subsequently to their adjacent nitrogens. The signal transfer stops in the absence of a ^{13}C or ^{15}N label. Prior to high-sensitivity proton detection, the pathway of the magnetization transfer is reversed.

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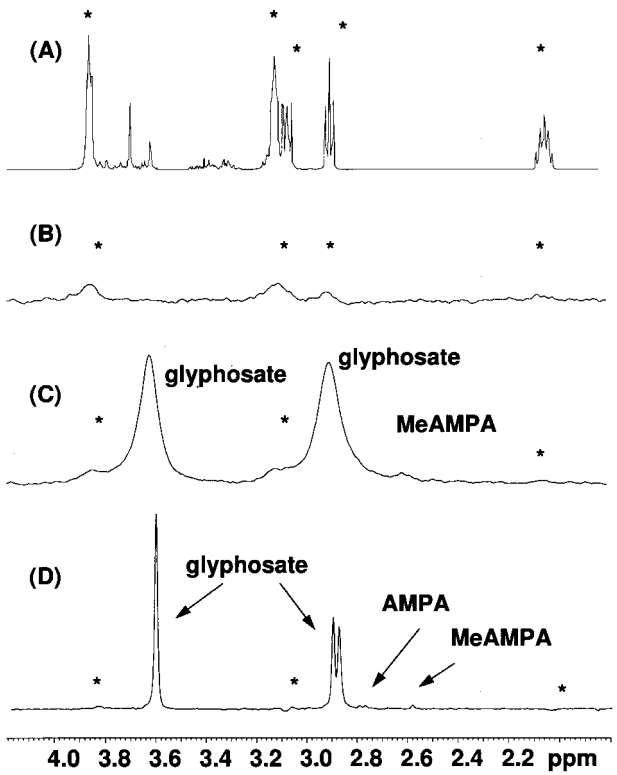

Figure 1. ^{1}H, ^{2}H, and TRIED experiments from an incubation of LBAA with triple-labeled glyphosate (^{13}C–^{15}N–^{13}C-labeled, >98% enriched) and d_4 glyphosate, are shown in (A), (C) and (D), respectively. The ^{2}H spectrum of the Dworkin–Foster/MOPS mixed incubation medium alone, is shown in (B). Glyphosate and AMPA-type metabolites are seen in both the ^{2}H and TRIED spectra of the medium sampled after 33 h incubation. MOPS peaks are marked by asterisks.

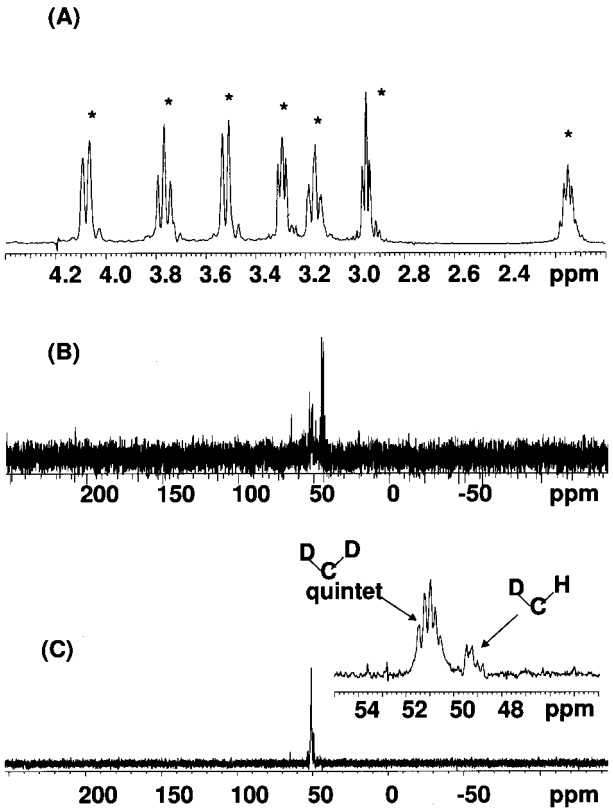

Figure 2. (A) ^{1}H spectrum of ~15 mg dideuterated, ^{13}C–^{15}N–^{13}C glyphosate (75% enriched at the carboxymethylene protons) in 80 mM MOPS buffer in D_2O. MOPS peaks are marked by asterisks. MOPS also dominates the 16 transient ^{13}C spectrum (B), but is completely filtered out in the refocused [^{2}H–^{13}C] INEPT spectrum (with proton decoupling) shown in (C). An inset of the refocused glyphosate peaks is also shown. The expected ^{2}H–^{13}C quintet is observed (J_{HC} = 30.7 Hz), as are resonances resulting from incompletely deuterated material. A modified version of the pulse sequence of Rinaldi *et al* was used,[4] and experimental condition were optimized according to the methodology of Schenker and von Philipsborn.[5]

Whereas 1.108% of all molecular bonds possess the ^{1}H–^{13}C label, only 0.0041% possess the ^{13}C–^{15}N label. This combination of isotope-editing and proton detection selectively cancels all but the desired signals arising from ^{13}C–^{15}N fragments which result from isotopically enriched starting material or its metabolites.

Although LBAA grew well in the initial Dworkin–Foster buffer system used, other organisms required different buffer mixtures to sustain growth.[2] These studies rapidly revealed physical interactions between the biological buffer used to nurture the microbes and the metabolites under investigation, compromising TRIED's ability to filter metabolite signals from the matrix.

To circumvent these problems, directly detected deuterium [^{2}H]NMR was investigated as one alternative. [^{2}H]NMR exhibits minimal sensitivity to background interference, as this nucleus has a natural abundance of 0.015%. Major metabolites, such as AMPA, are easily detected, but the concentration of minor metabolites was found to be comparable to the level of the ^{2}H background in the buffers used.

Deuterium–carbon [^{2}H–^{13}C] INEPT was also investigated. The experiment transfers magnetization from deuterium to carbon followed by carbon detection. This pathway yields high selectivity similar to TRIED: only 0.00017% of all naturally occurring molecules possess the labeled ^{2}H–^{13}C bond. Although not as sensitive as the proton-detected TRIED, this methodology utilizes the additional advantage of the wide chemical shift dispersion of carbon with minimal sensitivity to background matrix effects.

A ^{1}H spectrum of metabolites produced by LBAA after 33 h incubation with ^{13}C–^{15}N–^{13}C-labeled glyphosate in half MOPS medium illustrates interferences of the background matrix signals in conventional spectra, Fig 1A.[3] In this spectrum, the large number of resonances, combined with a high degree of signal overlap, make straightforward identification of peaks due to glyphosate and its metabolites impossible. The intense contaminating multiplets in the proton spectrum at ~2.9 ppm completely obscure the methylene in the glyphosate spectrum, yet are suppressed in the TRIED spectrum (D). The latter spectrum, while exhibiting some sensitivity to the concentration of MOPS, shows resonances from

glyphosate, AMPA, and *N*-methylAMPA (MeAMPA), having filtered out interfering signals from unlabeled molecules. MOPS peaks are comparable in size to those of the low concentration metabolites, as seen by comparison of the ^{2}H spectrum of a sample of the mixed Dworkin–Foster/MOPS medium alone (B) with that of the 33-h incubation (C). This background signal led to difficulties in definitively assigning metabolites in the deuterium spectrum, as did uncancelled MOPS peaks in the TRIED spectrum. [^{2}H–^{13}C] INEPT was utilized in an attempt to minimize interactions by combining magnetization transfer from deuterium to carbon.

An 80 mM MOPS solution in D_2O of approximately 15 mg of dideuterated glyphosate (^{13}C–^{15}N–^{13}C-labeled, 75% ^{2}H-enriched at the carboxymethylene protons) was prepared and studied by (A) proton, (B) proton, and (C) [^{2}H–^{13}C] INEPT, Fig 2. MOPS is again the dominant feature of both the proton and carbon spectra. In the [^{2}H–^{13}C] INEPT spectrum, however, only resonances resulting from the carboxymethylene of the glyphosate are detected. The MOPS peaks have been filtered out, leaving only the resonances of the desired compound. [^{2}H–^{13}C] INEPT has a theoretical molar cancellation efficiency of greater than 5×10^{5}:1 in addition to the increased chemical shift dispersion of carbon. Together, TRIED, [^{2}H]NMR and [^{2}H–^{13}C] INEPT form a powerful arsenal of isotope-edited techniques for the study of glyphosate metabolism in extracts from biological systems.

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Approaches to refining pesticide risk assessments – the spatial estimation of potential leaching risk

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Abstract: A Geographic Information System (GIS) has been combined with a simple leaching model to characterize the factors that influence pesticide leaching, and to identify the spatial distribution of these factors. The results were compared with those of a conventional simulation modeling approach, and a strong correlation was found for 40 selected sites in central and eastern USA.

Keywords: pesticide leaching; geographic information system; production

Addressing the landscape-wide risk of pesticide leaching in areas of chemical-intensive production has been one of the significant recent research topics in agriculture. Although simulation models are commonly used, the spatial variation in pesticide leaching cannot adequately be described on a large scale by such models. Kellogg *et al*[1] and Battaglin and Goolsby[2] developed a US nationwide map of ground-water vulnerability by using a Geographic Information System (GIS) which integrated information on hydrology, major resource areas, federal lands, and county boundaries. A GIS (ESRI Arc/Info, 1996) is a computer analysis and mapping system that was designed for data retrieval, storage, analysis and data presentation. GIS offers a tool for spatial analysis while simulation modeling provides a means for assessment of potential transport through the soil profile at a specific location. The integration of GIS and simulation modeling becomes useful and inevitable in risk assessment at a landscape scale.[3]

This study focused on combining a simple leaching model with GIS to characterize the factors that influence leaching and to identify the spatial distribution of these factors, and then validated the results against simulation models. The study objectives were to investigate and develop GIS tools to examine the environmental fate and exposure arising from use of Zeneca products, to verify GIS relative leaching predictions using Pesticide Root Zone Model[4] (PRMZ2) outputs, and to identify areas with a high risk of leaching of herbicides.

To test the utility of the tools, we selected a potentially mobile herbicide. We applied a GIS map overlay, simple leaching ranking criteria for precipitation and temperature, a soil leaching screening model,[5] statistics, and Kriging geostatistics. Datasets used included STATSGO (NRSC, USDA), cropping agricultural statistics (USDA, 1992) and weather information (Earth Info, Inc.).

The analysis of chemical dissipation and environmental fate indicated the following four principal factors that affect chemical fate: cropping systems, soil-water related properties, precipitation and temperature. By using detailed knowledge of the environment behavior of the herbicide and appropriately weighting and then combining these factors contributing to leaching risk, it was possible to define an overall leaching risk index (OLR). Three of the principal factors were weighted as follows: soil